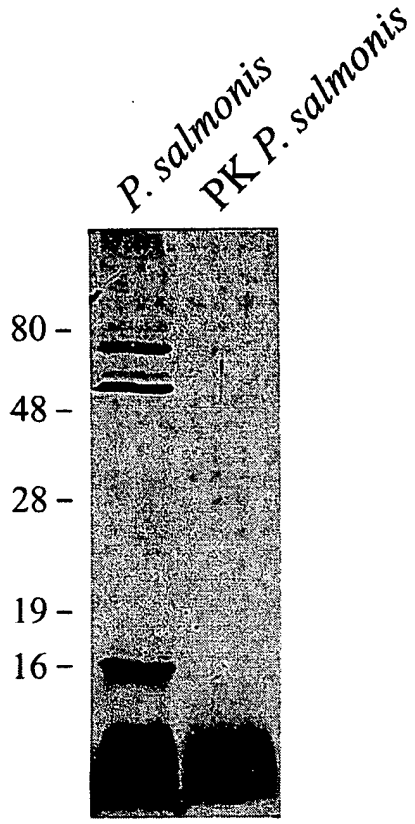
Susan Alpert Siegel, Ph.D.
Klarquist Sparkman, LLP
121 SW Salmon Street, Suite 1600
Portland, Oregon 97204
Telephone: (503) 226-7391
Express Mail Label No. EV331581445US
Date of Deposit: February 18, 2004

FIGURE 1. WESTERN BLOT ANALYSIS OF *P. SALMONIS*



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Klarquist Sparkman, LLP
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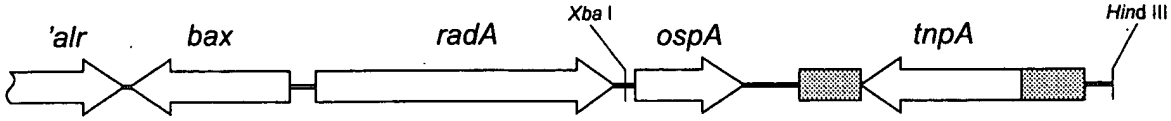
FIGURE 2

A. ORF's in the region of the *ospA* gene from *P. salmonis*



B. DNA sequence of *ospA* gene from *P. salmonis* (SEQ ID:1)

ATGAACAGAGGATGTTTGCAAGGTAGTAGTCTAATTATTATCAGTGTGTTTTTAGTTGGCTGTGCCCAGA
ACTTTAGTCGTCAAGAAGTCGGAGCTGCGACTGGGGCTGTTGTTGGCGGTGTTGCTGGCCAGCTGTTTGG
TAAAGGTAGTGGTCGAGTTGCAATGGCCATTGGTGGTGCTGTTTTGGGTGGATTAATTGGTTCTAAAATC
GGTCAATCGATGGATCAGCAGGATAAAATAAAGCTAAACCAGAGTTTGGAAAAGGTAAAAGCAGGGCAAG
TGACACGTTGGCGTAATCCAGATACAGGCAATAGTTATAGTGTTGAGCCAGTGCGTACTTACCAGCGTTA
CAATAAGCAAGAGCGTCGCCAGCAATATTGTCGAGAATTTCAGCAAAAGGCGATGATTGCAGGGCAGAAG
CAAGAGATTTACGGCACTGCATGCCGGCAACCGGATGGTCGTTGGCAAGTCATTTCAACAGAAAAA

Amino acid sequence of OspA protein (SEQ ID:2)

MNRGCLQGSSLIIISVFLVGCAQNFSRQEVGAATGAVVGGVAGQLFGKGSGRVAMAIGGAVLGGLIGSKI
GQSMDQQDKIKLNQSLEKVKAGQVTRWRNPDTGNSYSVEPVRTYQRYNKQERRQQYCREFQQKAMIAGQK
QEIYGTACRQPDGRWQVISTEK

C. Sequence alignment of the OspA proteins of *P. salmonis* and *R. prowazekii*

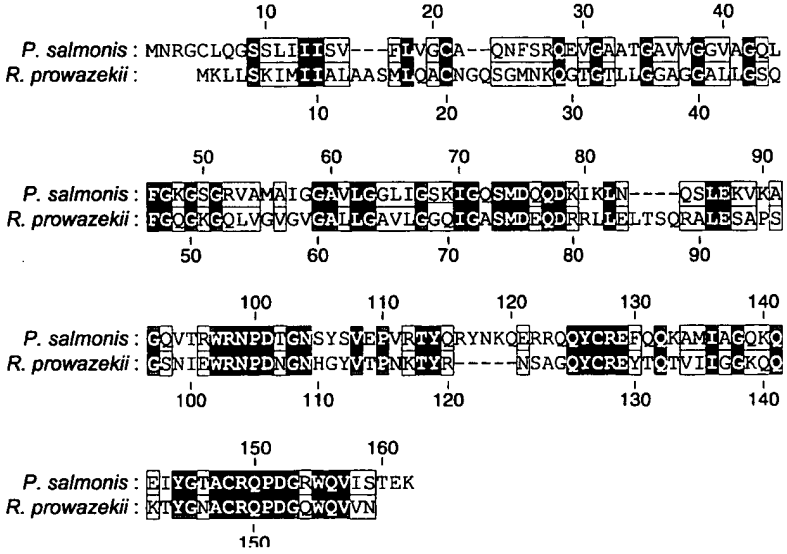


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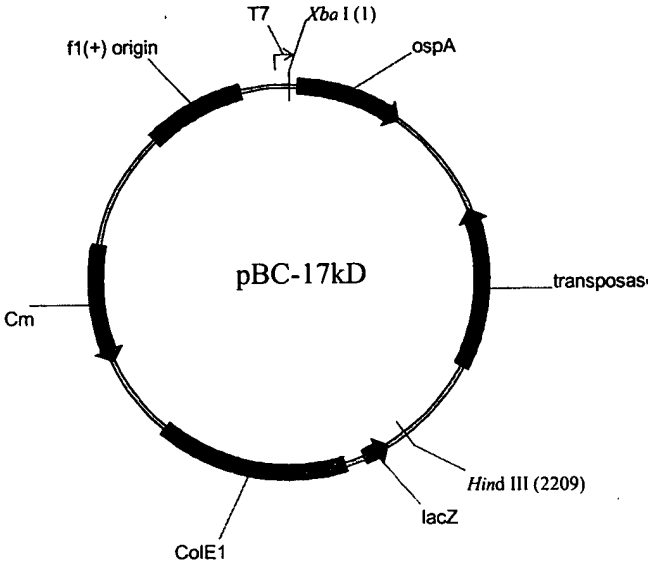
FIGURE 3

A. Map of plasmid pBC-17kDa encoding the *ospA* ORF.



B. Western blot analysis of OspA expression.

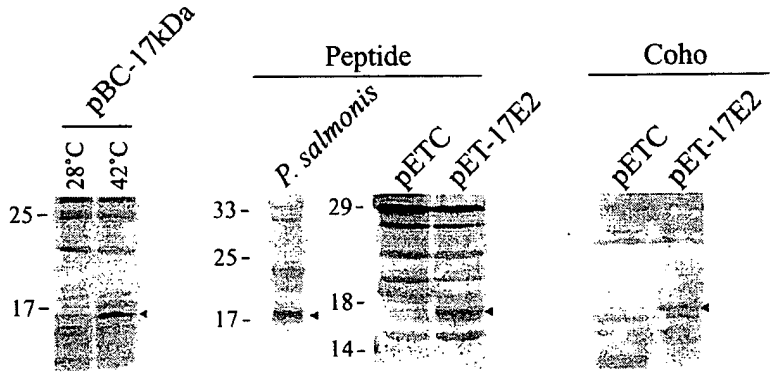


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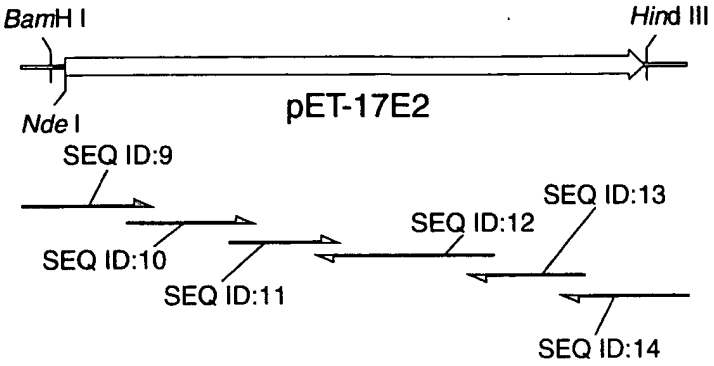
FIGURE 4.

A. Strategy for construction of the *E. coli* codon optimized *ospA* gene.



B. Oligonucleotide #1 (SEQ ID:9)

CGCCAGGGTTTTCCCAGTCACGACGGATCCGTCTCATATGCGTGGTTGCCTGCAGGGCAGCTCTCTGATC
ATTATCTCTGTTTTCCTGGTGGGTTGCGCCCAGAACTTCAG

Oligonucleotide #2 (SEQ ID:10)
TGGGTTGCGCCCAGAACTTCAGCCGCCAGGAAGTTGGCGCGGCCACCGGTGCGGTTGTGGGCGGTGTTGC
CGGCCAGCTGTTCGGTAAAGGCTCTGGTCGTGTGGCGATG

Oligonucleotide #3 (SEQ ID:11)
AAAGGCTCTGGTCGTGTGGCGATGGCCATCGGCGGTGCGGTTCTGGGCGGTCTGATTGGCTCTAAAATCG
GTCAGAGCATGGACCAGCAGGATA

Oligonucleotide #4 (SEQ ID:12)
GTTCCACAGAGTAGCTGTTACCGGTGTCCGGATTACGCCAACGAGTAACCTGGCCGGCTTTCACTTTTTC
CAGAGACTGGTTCAGTTTGATTTTATCCTGCTGGTCCATGCTCTGACC

Oligonucleotide #5 (SEQ ID:13)
GGTGCCGTAGATTTCCTGTTTCTGACCTGCGATCATGGCTTTCTGCTGAAATTCGCGGCAGTACTGCTGA
CGGCGTTCCTGTTTGTTGTAACGCTGGTAGGT

Oligonucleotide #6 (SEQ ID:14)
CGTCCTCTCGTCCTGGTCCGAATTCAGATAAGCTTATTTTTCGGTGCTAATCACCTGCCAGCGGCCATCC
GGCTGACGGCACGCGGTGCCGTAGATTTCCTGTTTCTGAC

C. DNA sequence of *E. coli* optimized *ospA* gene, 17e2 (SEQ ID:3)

ATGCGTGGTTGCCTGCAGGGCAGCTCTCTGATCATTATCTCTGTTTTCCTGGTGGGTTGCGCCCAGAACT
TCAGCCGCCAGGAAGTTGGCGCGGCCACCGGTGCGGTTGTGGGCGGTGTTGCCGGCCAGCTGTTCGGTAA
AGGCTCTGGTCGTGTGTCGATGGCCATCGGCGGTGCGGTTCTGGGCGGTCTGATTGGCTCTAAAATCGGT
CAGAGCATGGACCAGCAGGATAAAATCAAACTGAACCAGTCTCTGGAAAAAGTGAAAGCCGGCCAGGTTA
CTCGTTGGCGTAATCCGGACACCGGTAACAGCTACTCTGTGGAACCGGTTCGCACCTACCAGCGTTACAA
CAAACAGGAACGCCGTCAGCAGTACTGCCGCGAATTTCAGCAGAAAGCCATGATCGCAGGTCAGAAACAG
GAAATCTACGGCACCGCGTGCCCTCAGCCGGATGGCCGCTGGCAGGTGATTAGCACCGAAAAA

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FIGURE 5

A. Amino acid sequence of optimized OspA protein, 17E2, (SEQ ID:4).

MRGCLQGSSLIIISVFLVGCAQNFSRQEVGAATGAVVGGVAGQLFGKGSGRVSMAIGGAVLGGLIGSKIG
QSMDQQDKIKLNQSLEKVKAGQVTRWRNPDTGNSYSVEPVRTYQRYNKQERRQQYCREFQQKAMIAGQKQ
EIYGTACPQPDGRWQVISTEK

B. DNA sequence of c17e2 *ospA* construct with N-terminal fusion partner (SEQ ID:5).

ATGTCAGTTGAATTCTACAACTCTAACAAATCAGCACAAACAAACTCAATTACACCAATAATCAAAATTA
CTAACACATCTGACAGTGATTTAAATTTAAATGACGTAAAAGTTAGATATTATTACACAAGTGATGGTAC
ACAAGGACAAACTTTCTGGTGTGACCATGCTGGTGCATTATTAGGAAATAGCTATGTTGATAACACTAGC
AAAGTGACAGCAAACTTCGTTAAAGAAACAGCAAGCCCAACATCAACCTATGATACATATCTGGATCCGT
CTCATATGCGTGGTTGCCTGCAGGGCAGCTCTCTGATCATTATCTCTGTTTTCCTGGTGGGTTGCGCCCA
GAACTTCAGCCGCCAGGAAGTTGGCGCGGCCACCGGTGCGGTTGTGGGCGGTGTTGCCGGCCAGCTGTTC
GGTAAAGGCTCTGGTCGTGTGTCGATGGCCATCGGCGGTGCGGTTCTGGGCGGTCTGATTGGCTCTAAAA
TCGGTCAGAGCATGGACCAGCAGGATAAAATCAAACTGAACCAGTCTCTGGAAAAAGTGAAAGCCGGCCA
GGTTACTCGTTGGCGTAATCCGGACACCGGTAACAGCTACTCTGTGGAACCGGTTCGCACCTACCAGCGT
TACAACAAACAGGAACGCCGTCAGCAGTACTGCCGCGAATTTCAGCAGAAAGCCATGATCGCAGGTCAGA
AACAGGAAATCTACGGCACCGCGTGCCCTCAGCCGGATGGCCGCTGGCAGGTGATTAGCACCGAAAAA

C. Amino acid sequence of C17E2 OspA construct with N-terminal fusion partner (SEQ ID:6).

MSVEFYNSNKSAQTNSITPIIKITNTSDSDLNLNDVKVRYYYTSDGTQGQTFWCDHAGALLGNSYVDNTS
KVTANFVKETASPTSTYDTYLDPSHMRGCLQGSSLIIISVFLVGCAQNFSRQEVGAATGAVVGGVAGQLF
GKGSGRVSMAIGGAVLGGLIGSKIGQSMDQQDKIKLNQSLEKVKAGQVTRWRNPDTGNSYSVEPVRTYQR
YNKQERRQQYCREFQQKAMIAGQKQEIYGTACPQPDGRWQVISTEK

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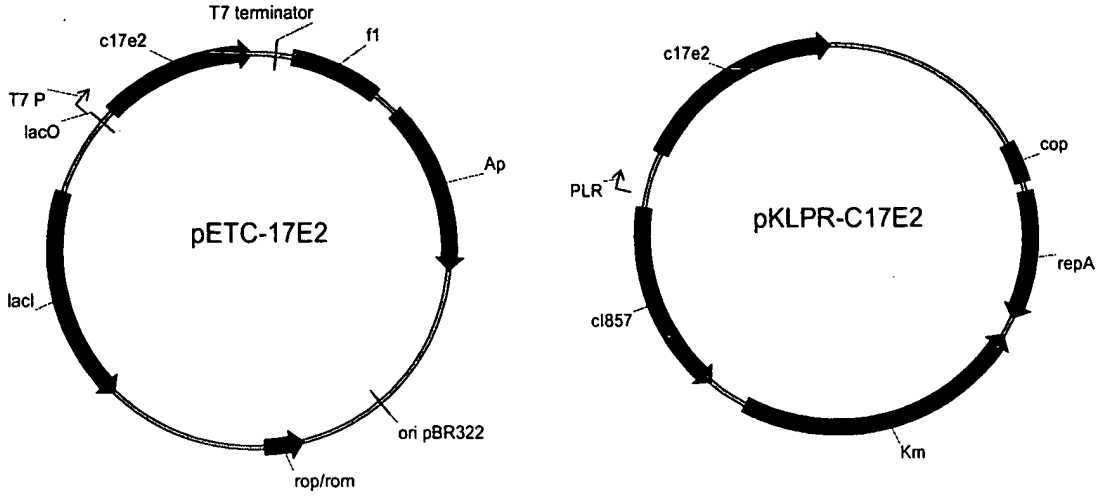
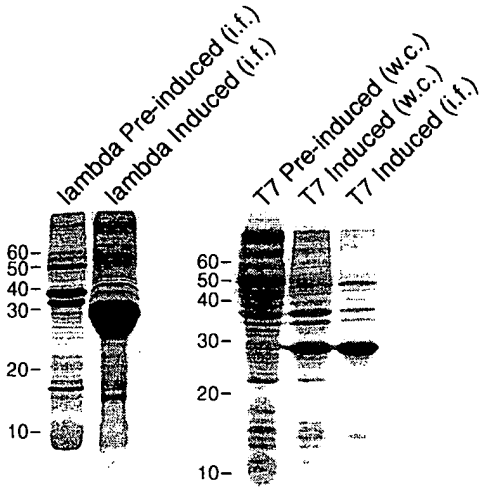
FIGURE 6

A. Expression vectors encoding the optimized *ospA* fusion constructs



B. SDS-PAGE analysis of C17E2 expression.



Susan Alpert Siegel, Ph.D.
Klarquist Sparkman, LLP
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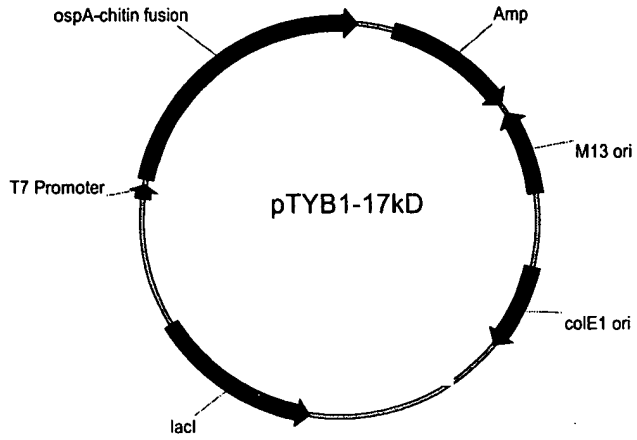
FIGURE 7

Map of the *ospA*-fusion construct encoding a C-terminal fusion partner under T7 promoter control.



Susan Alpert Siegel, Ph.D.
Klarquist Sparkman, LLP
121 SW Salmon Street, Suite 1600
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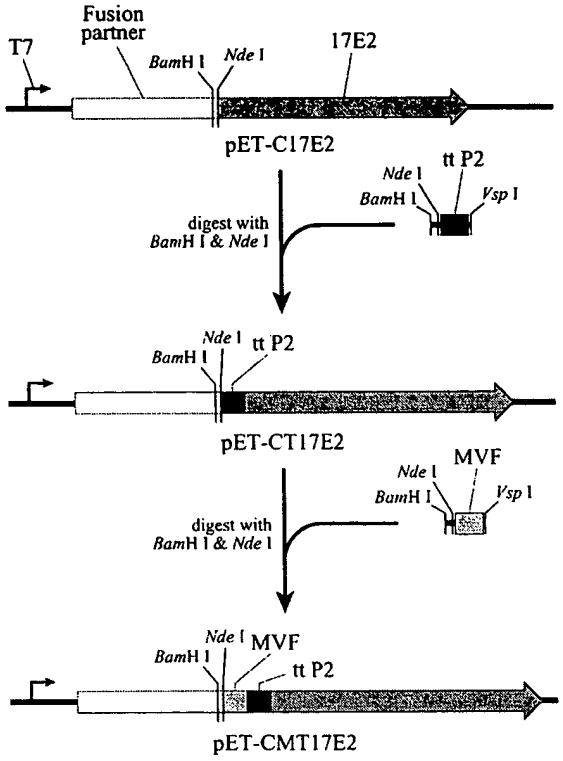
FIGURE 8

A. CLONING STRAGEGY FOR OSPA TCE FUSION PROTEIN CONSTRUCTS.



B. **(a) Nucleotide sequence of the tt P2 oligonucleotide (SEQ ID:17)**

CGCCAGGGTTTTCCCAGTCACGACGGATCCGTCTCATATG**CAGTACATTAAAGCAAACTCTAAATTCATC**
GGTATTACCGAACTGATTAATTAAGCTTCGGACCAGGACGAGAGGACG

(b) Nucleotide sequence of the MVF oligonucleotide (SEQ ID:18)

CGCCAGGGTTTTCCCAGTCACGACGGATCCGTCTCATATG**CTGTCTGAAATCAAAGGTGTTATCGTTCAT**
CGTCTGGAAGGCGTGATTAATTAAGCTTCGGACCAGGACGAGAGGACG

(c) Amino acid sequence of the tt P2 TCE (SEQ ID:19)

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(d) Amino acid sequence of the MVF TCE (SEQ ID:20)

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Klarquist Sparkman, LLP
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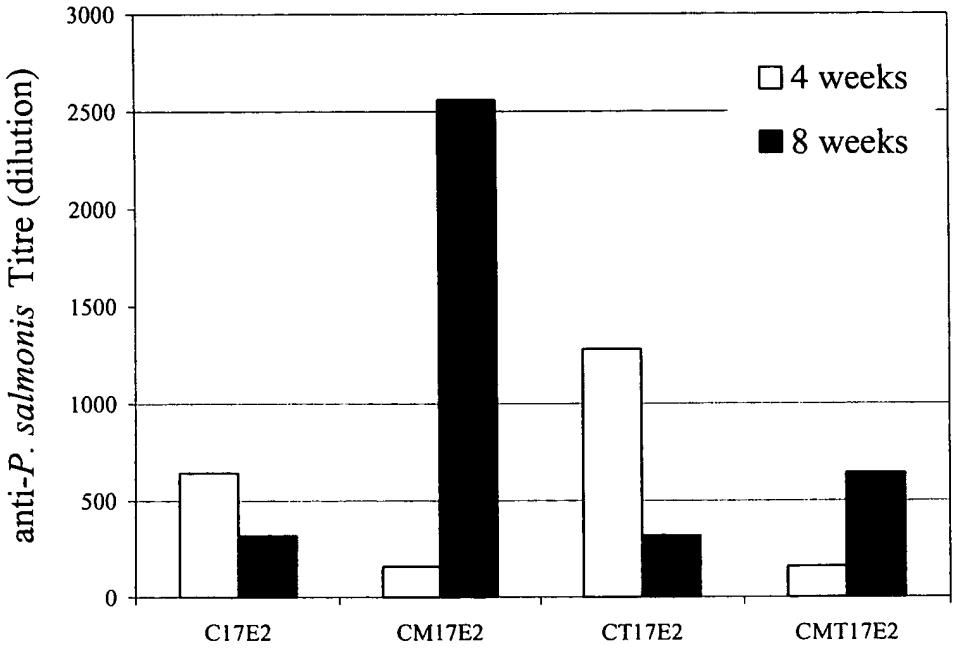
FIGURE 9

Coho salmon antibody titres against OspA-fusion protein candidate vaccines.



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Klarquist Sparkman, LLP
121 SW Salmon Street, Suite 1600
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FIGURE 10

Whole lymphocyte proliferative response to OspA-fusion proteins in Atlantic salmon.

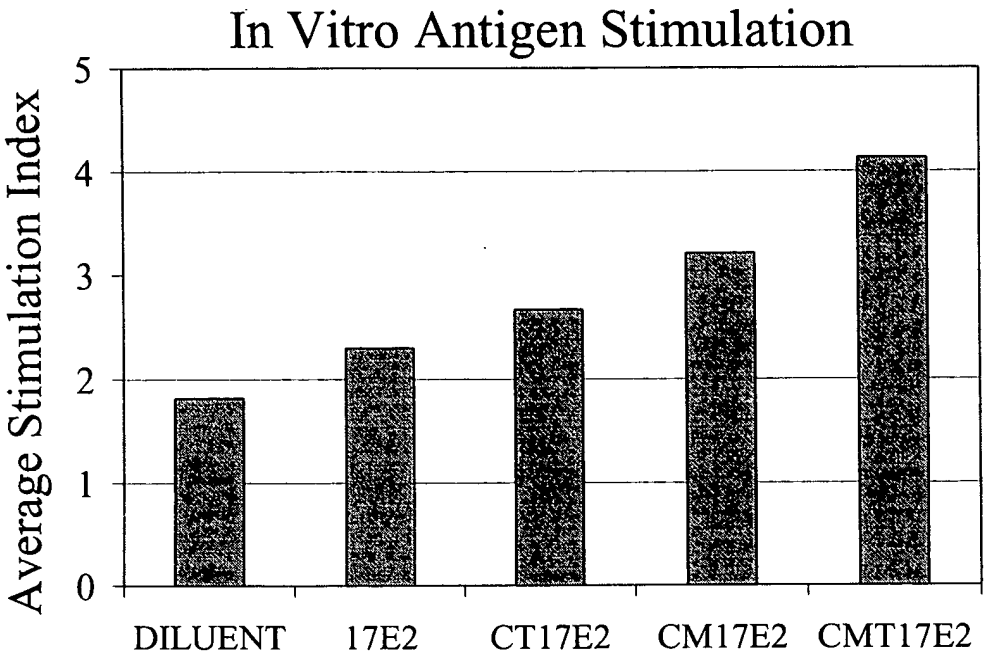

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FIGURE 11

Vaccine trial in coho salmon of OspA fusion proteins.